Amendments to the Specification

a. at pages 8-9

FIG. 1 is an elution pattern of a saccharide, obtained by α-isomaltosyl-transferring enzyme from a microorganism of the species *Bacillus ~~globiformis~~ globisporus* C9 strain, when determined on high-performance liquid chromatography.

FIG. 2 is a nuclear resonance spectrum (^{1}H-NMR) of cyclotetrasaccharide, obtained by the enzymatic reaction using α-isomaltosyl-transferring enzyme from a microorganism of the species *Bacillus globisporus~~globiformis~~* C9 strain.

FIG. 3 is a nuclear resonance spectrum (^{13}C-NMR) of cyclotetrasaccharide, obtained by the enzymatic reaction using α-isomaltosyl-transferring enzyme from a microorganism of the species *Bacillus globisporus~~globiformis~~* C9 strain.

FIG. 5 shows the thermal influence on the enzymatic activity of α-isomaltosylglucosaccharide-forming enzyme from a microorganism of the species *Bacillus globisporus~~globiformis~~* C9 strain.

FIG. 6 shows the pH influence on the enzymatic activity of α-isomaltosylglucosaccharide-forming enzyme from a microorganism of the species *Bacillus globisporus~~globiformis~~* C9 strain.

FIG. 7 shows the thermal stability of α-isomaltosylglucosaccharide-forming enzyme from a microorganism of the species *Bacillus globisporus~~globiformis~~* C9 strain.

FIG. 8 shows the pH stability of α-isomaltosylglucosaccharide-forming enzyme from a microorganism of the species *Bacillus globisporus~~globiformis~~* C9 strain.

FIG. 9 shows the thermal influence on the enzymatic activity of α-isomaltosyl-transferring enzyme from a microorganism of the species *Bacillus globisporus~~globiformis~~* C9 strain.

FIG. 10 shows the pH influence on the enzymatic activity of α-isomaltosyl-transferring enzyme from a microorganism of the species *Bacillus globisporus~~globiformis~~* C9 strain.

FIG. 11 shows the thermal stability of α-isomaltosyl-transferring enzyme from a microorganism of the species *Bacillus globisporus~~globiformis~~* C9 strain.

FIG. 12 shows the pH stability of α-isomaltosyl-transferring enzyme from a microorganism of the species *Bacillus globisporus~~globiformis~~* C9 strain.

b. at page 13

FIG. 50 is an x-ray diffraction spectrum for an anhydrous crystalline powder of the cyclotetrasaccharide~~, penta- to hexa-hydrate,~~ of the present invention, obtained by drying *in vacuo* at 40°C, when determined on x-ray powder diffraction analysis.

c. at page 44

as a coffee, cocoa, juice, carbonated beverage, sour milk beverage, and beverage containing a lactic acid bacterium; instant food products such as instant pudding mix, instant hot

cake mix, instant juice or soft drink, instant coffee, "*sokuseki-shiruko*" (an instant mix of adzuki-bean soup with rice cake), and instant soup mix; and other foods and beverages such as solid foods for babies, foods for therapy, health/tonic drinks, peptide foods, and frozen foods. The cyclotetrasaccharide and the saccharide compositions comprising the same of the present invention can be arbitrary used to prolong or retain the flavor and taste of fresh-baked Japanese and Western confectioneries and to improve the taste preference of feeds and pet foods for animals and pets such as domestic animals, poultry, honey bees, silk ~~warms~~worms, and ~~fishes~~fish; and also they can be arbitrary arbitrarily used as a sweetener, taste-improving agent, flavoring substance, quality-improving agent, and stabilizer in other products in a paste or liquid form such as a tobacco, cigarette, tooth paste, lipstick, rouge, lip cream, internal liquid medicine, tablet, troche, cod liver oil in the form of drop, cachou, oral refrigerant, gargle, cosmetic, and pharmaceutical. When used as a quality-improving agent or stabilizer, the cyclotetrasaccharide and the saccharide compositions comprising the same of the present invention can be arbitrarily used in biologically active substances susceptible to lose their effective ingredients and activities, as well as in health foods and pharmaceuticals containing the biologically active substances. Examples of such biologically active substances are liquid preparations containing lymphokines such as α-, β- and γ-interferons, tumor necrosis factor-α (TNF-...

d. at page 46

α), tumor necrosis factor-β (TNF-β), macrophage migration inhibitory factor, colony-stimulating factor, transfer factor, and interleukin 2; liquid preparations containing hormones such as insulin, growth hormone, prolactin, erythropoietin, and

follicle-stimulating hormone; biological preparations such as BCG vaccine, Japanese encephalitis vaccine, measles vaccine, live polio vaccine, smallpox vaccine, tetanus toxoid, Trimeresurus antitoxin, and human immunoglobulin; antibiotics such as penicillin, erythromycin, chloramphenicol, tetracycline, streptomycin, and kanamycin sulfate; liquid preparations containing vitamins such as thiamine, riboflavin, L-ascorbic acid, cod liver oil, carotenoid, ergosterol, and tocopherol; highly unsaturated fatty acids and ester derivatives thereof such as EPA, DHA, and arachidonic acid; solutions of enzymes such as lipase, elastase, urokinase, protease, β-amylase, isoamylase, glucanase, and lactase; extracts such as ginseng extract, snapping turtle extract, chlorella extract, aloe extract, and propolis extract; and royal jelly. By using the cyclotetrasaccharide and the saccharide compositions comprising the same of the present invention, the above biologically active substances and other pastes of living microorganisms such as viruses, lactic acid bacteria, and yeasts can be ~~arbitrary~~ arbitrarily prepared into health foods and pharmaceuticals in a liquid, paste, or solid form, which have a satisfactorily-high stability and quality with less fear of losing or inactivating their effective ingredients and activities.

e. at page 46-47, paragraph 2

Similarly as other naturally occurring saccharides, since the cyclotetrasaccharide and the saccharide compositions comprising the same of the present invention quite scarcely stimulate the skin when applied thereupon and effectively retain the moisture in the skin, they can be advantageously incorporated into external dermal compositions for use. In the external dermal compositions, the cyclotetrasaccharide and the saccharide compositions comprising the same of the present

invention can be usually used in an appropriate combination with one or more dermatologically applicable other ingredients of oils and lipids, waxes, hydrocarbons, fatty acids, esters, alcohols, surfactants, dyes, flavors, hormones, vitamins, plant extracts, animal extracts, microbial extracts, salts, ultraviolet absorbents, photosensitizing dyes, antioxidants, antiseptics/bactericides, antiperspirants/deodorants, refreshments, chelating agents, skin whitening agents, anti-~~inflamatories~~inflammatories, enzymes, saccharides, amino acids, and thickening agents. For example, in the field of cosmetics, the external dermal compositions can be provided in the form of a lotion, cream, milky lotion, gel, powder, paste, or block, for example, cleaning cosmetics such as soaps, cosmetic soaps, washing powders for the skin, face washing creams, facial rinses, body shampoos, body rinses, shampoos, and powders for washing hair; cosmetics for hair such as set lotions, hair blows, stick pomades, hair creams, pomades, hair sprays, hair liquids, hair tonics, hair lotions, hair restorers, hair dyes, treatments for scalp, hair cosmetics, gloss-imparting hair oils, hair oils, and combing oils; base cosmetics such as cosmetic lotions, vanishing creams, emollient creams, emollient lotions, cosmetic packs in the form of a jelly ~~peal~~ peel off, jelly wiping, paste washing, powders, cleansing creams, cold creams, hand creams, hand lotions, milky lotions, moisture-imparting liquids, after/before shaving lotions, after shaving creams, after shaving foams, before shaving creams, and baby oils; makeup cosmetics such as foundations in the form of a liquid, cream or solid, talcum powders, baby powders, body powders, perfume powders, makeup bases, powders in the form of a cream, paste, liquid, solid or powder, eye shadows, eye creams, mascaras, eyebrow pencils, eyelash makeups, rouges, rouge lotions; perfume cosmetics such as perfumes, paste/powder

perfumes, *eau de Cologne*s, perfume *Cologne*s, and *eau de toilette*; suntan and suntan preventive cosmetics such as suntan creams, suntan lotions, and suntan oils; nail cosmetics such as manicures, pedicures, nail colors, nail lacquers, and nail makeup materials; eyeliner cosmetics; rouges and lipsticks such as lipsticks, lipcreams, paste rouges, and lip-glosses; oral cosmetics such as tooth pastes and mouth washes; and bath cosmetics such as bath salts/oils, and bath cosmetic materials. In the field of pharmaceuticals, the external dermal compositions can be provided in the form of a wet compresses, sprays, applications, bath agents, sticking agents, ointments, pastes, embrocations, lotions, and cataplasms.

e. at page 49

alcohol, cetanol, setostearyl alcohol, stearyl alcohol, oleyl alcohol, behenyl alcohol, ~~lanoline~~ lanolin alcohol, hydrogenated ~~lanoline~~ lanolin alcohol, hexyldecanol, octyldodecanol, and polyethylene glycol; lower alcohols including polyalcohols such as ethanol, propanol, isopropanol, butanol, ethylene glycol, propylene glycol, and glycerine; and derivatives thereof.

Examples of the esters usable in the present invention are hexyl laurate, isopropyl myristate, myristyl myristate, cetyl myristate, octyl dodecyl myristate, isopropyl palmitate, butyl stearate, cholesteryl stearate, cholesteryl acetate, cholesteryl n-lactate, cholesteryl caproate, cholesteryl laurate, cholesteryl myristate, cholesteryl palmitate, cholesteryl stearate, cholesteryl 12-hydroxystearate, decyl oleate, octyldodecyl oleate, isopropyl ~~lanoline~~ lanolin fatty acid, glycerine trimyristate, propylene glycol dioleate, myristyl lactate, cetyl lactate, ~~lanoline~~ lanolin acetate, hexyldecyl dimethyloctanoate, and derivatives thereof.

The surfactants usable in the present invention are, for example, ~~anion~~ anionic surfactants such as zinc laurate, zinc myristate, zinc palmitate, magnesium stearate, sodium lauryl sulfate, sodium polyoxyethylene laurylether sulfate, triethanolamine polyoxyethylene laurylether sulfate, polyoxyethylene cetylether phosphate, polyoxyethylene alkylphenylether phosphate, sodium N-lauroyl sarcosinate, coconut fatty acid sarcosinate triethanolamine, coconut fatty acid sodium methyltaurate, and soybean phospholipid; ~~cation~~ cationic surfactants such as stearyltrimethylammonium chloride, distearyldimethylammonium chloride, benzalkonium chloride, cetylpyridinium chloride, alkylisoquinolinium bromide, and...

f. at page 51

The fragrances~~flavors~~ used generally in external dermal uses can be roughly classified into natural plant and animal fragrances, synthetic fragrances~~flavors~~, and mixtures thereof in an appropriate combination. Examples of the animal fragrances~~flavors~~ include musk, civetone, and ambergris. The plant fragrances~~flavors~~ are, for example, distillations, i.e., essential oils, obtainable by distilling, for example, with water vapor anise seeds, basil leaves, caraway fruit, cinnamon barks, coriander seeds, lavender flowers, nutmeg seeds, peppermint leaves, rose flowers, rosemary flowers, seeds, and leaves, and thyme leaves; extracts classified generally into absolutes, resinoids, oleo resins, and tinctures depending on properties and processes. Examples of the synthetic fragrances are acetophenone, anisole, benzyl alcohol, butyl acetate, camphor, citral, citronellol, cuminaldehyde, estragol, ethylvaniline, geranyl acetate, linarol, menthol, methyl p-cresol, methyl salicylate, phenyl acetate, vanillin, and derivatives thereof. In the present invention, fragrance~~flavor~~

compositions mixed with the aforesaid flavors in an appropriate combination can be arbitrarily used.

g. at page 52

Examples of the plant extracts usable in the present invention are, in addition to the aforesaid plant extracts used as ~~flavors~~fragrances, extracts such as those of chamomile, sage, aloe, scarlet sage, *Angelica keiskei*, avocado, nettle, fennel, oolong tea, coak tree bark, barley, *Abelmoschus esculentus*, allspice, seaweed, chinese quince, licorice, quince seed, gardenia, *Sasa albo-marginata*, cinnamon, black tea, rice bran, fermented rice bran, *Stevia rebaudiana*, celery, Japanese green gentian, soy bean, thyme, tea, common camellia, *Ligusticum acutilobum*, corn, carrot, *Rosa rugosa*, *hinoki* (Japanese cypress), dishcloth gourd, safflower, pine, peach, eucalyptus, creeping saxifrage, *yuzu* (citron), lily, Job's tears, Mugwort, *Cyanophta* (blue-green algae), seaweed, apple, *Serratia marcescens*, and lettuce; and compounds isolated from plants such as hinokitiol, azulene, chlorophyll, and glycyrrhizin. The animal extracts usable in the present invention include placenta extracts.

g. at page 54

Examples of the aseptics and bactericides usable in the present invention include, in addition to the aforesaid compounds with aseptic or ~~bactericide~~ bactericidal activities, phenol compounds such as phenol, p-chloro metacresol, resorcin, p-oxy benzoate, and cresol; acid compounds including those in a salt form such as benzoic acid, sorbic acid, salicylic acid, and boric acid; bisphenol halides such as hexachlorophene,

bithionol, and dichlorophene; amides such as 3,4,4'-trichlorocarvaniride, undecylenic acid monoethanolamide; quaternary ammonium compounds such as benzalkonium chloride, benzethonium chloride, and decalinium chloride; chlorhexidine hydrochloride, 1-hydroxypyridine-2-thione, lysozyme chloride; and derivatives thereof.

h. at page 71

As evident ~~form~~ from the results in Table 3, the enzyme activity was greatly inhibited by Hg^{2+}, Cu^{2+}, and EDTA, and was also inhibited by Ba^{2+} and Sr^{2+}. It was also found that the enzyme was activated by Ca^{2+} and Mn^{2+}.

i. at page 73

As evident ~~form~~ from the results in Table 4, the enzyme activity was greatly inhibited by Hg^{2+} and was also inhibited by Cu^{2+}. It was also found that the enzyme was not activated by Ca^{2+} and not inhibited by EDTA.

j. at page 79

A ~~faction~~ fraction of α-isomaltosyl-transferring enzyme, which had been separated from a fraction with α-isomaltosylglucosaccharide-forming enzyme by the affinity chromatography in Experiment 7-1, was dialyzed against 10 mM phosphate buffer (pH 7.0) containing 1 M ammonium sulfate. The dialyzed solution was centrifuged to remove insoluble impurities, and the resulting supernatant was fed to hydrophobic chromatography using 350 ml of "BUTYL-TOYOPEARL 650 M", a gel commercialized by Tosoh Corporation, Tokyo, Japan. The enzyme adsorbed on the gel and then it was eluted at about 0.3 M

ammonium sulfate when eluted with a linear gradient decreasing from 1 M to 0 M of ammonium sulfate, followed by collecting fractions with the enzyme activity. The fractions were pooled and dialyzed against 10 mM phosphate buffer (pH 7.0) containing 1 M ammonium sulfate. The resulting dialyzed solution was centrifuged to remove impurities and fed to affinity chromatography using "SEPHACRYL HR S-200" gel to purify the enzyme. The amount of enzyme activity, specific activity, and yield of the α-isomaltosyl-transferring enzyme in each purification step are in Table 6.

k. at page 83

As evident ~~form~~ from the results in Table 7, the enzyme activity was greatly inhibited by Hg^{2+}, Cu^{2+}, and EDTA and was also inhibited by Ba^{2+} and Sr^{2+}. It was also found that the enzyme was activated by Ca^{2+} and Mn^{2+}.

l. at page 85

As evident ~~form~~ from the results in Table 8, the enzyme activity was greatly inhibited by Hg^{2+} and was also inhibited by Cu^{2+}. It was also found that the enzyme was not activated by Ca^{2+} and not inhibited by EDTA.

m. at page 87

~~Table 9~~

~~Peptide name~~	~~Internal partial amino acid sequence~~
~~P64~~	~~aspartic acid alanine serine alanine asparagine valine threonine threonine~~
~~P88~~	~~tryptophane serine leucine glycine phenylalanine methionine asparagine phenylalanine~~
~~P99~~	~~asparagine tyrosine threonine aspartic acid alanine tryptophane methionine phenylalanine~~

Please replace Table 9 on page 87 with the following amended table:

Table 9

Peptide name	Internal partial amino acid sequence
P64	aspartic acid-alanine-serine-alanine-asparagine-valine-threonine-threonine
P88	tryptophan-serine-leucine-glycine-phenylalanine-methionine-asparagine-phenylalanine
P99	asparagine-tyrosine-threonine-aspartic acid-alanine-tryptophan-methionine-phenylalanine

n. at page 88

~~Table 10~~

~~Peptide name~~	~~Internal partial amino acid sequence~~
~~P22~~	~~glycine-asparagine-glutamic acid-methionine-arginine-asparagine-glutamine-tyrosine~~
~~P63~~	~~isoleucine-threonine-threonine-tryptophane-proline-isoleucine-glutamic acid-serine~~

~~(Continued)~~

~~Peptide name~~	~~Internal partial amino acid sequence~~
~~P71~~	~~tryptophane-alanine-phenylalanine-glycine-leucine-tryptophane-methionine-serine~~

Please replace Table 10 on page 87 with the following amended table:

Table 10

Peptide name	Internal partial amino acid sequence
P22	glycine-asparagine-glutamic acid-methionine-arginine-asparagine-glutamine-tyrosine
P63	isoleucine-threonine-threonine-tryptophan-proline-isoleucine-glutamic acid-serine

(Continued)

Peptide name	Internal partial amino acid sequence
P71	tryptophan-alanine-phenylalanine-glycine-leucine-tryptophane-methionine-serine

o. at page 99

As evident ~~form~~ from the results in Table 13, the enzyme activity was greatly inhibited by Hg^{2+}, Cu^{2+}, and EDTA. It was also found that the enzyme was activated by Ca^{2+} and Mn^{2+}.

p. at page 101

As evident ~~form~~ from the results in Table 14, the enzyme activity was greatly inhibited by Hg^{2+} and was also inhibited by Cu^{2+}. It was also found that the enzyme was not activated by Ca^{2+} and not inhibited by EDTA.

q. at page 105

~~Table 16~~

~~Peptide name~~	~~Internal partial amino acid sequence~~
~~PN21~~	~~asparagine tryptophane tryptophane methionine serine lysine~~
~~PN38~~	~~threonine aspartic acid glycine glycine glutamic acid methionine valine tryptophane~~
~~PN69~~	~~asparagine isoleucine tyrosine leucine proline glutamine glycine aspartic acid~~

Please replace Table 16 on page 105 with the following amended table:

Table 16

Peptide name	Internal partial amino acid sequence
PN21	asparagine-tryptophan-tryptophan-methionine-serine-lysine
PN38	threonine-aspartic acid-glycine-glycine-glutamic acid-methionine-valine-tryptophane
PN69	asparagine-isoleucine-tyrosine-leucine-proline-glutamine-glycine-aspartic acid

r. at page 114, paragraph 1

As evident ~~form~~ from the results in Table 19, it was revealed that the enzyme activity was greatly inhibited by Hg^{2+}, Cu^{2+} and EDTA.

s. at page 120-121, paragraph 3

As evident ~~form~~ from the results in Table 21, it was revealed that the enzyme activity was greatly inhibited by Hg^{2+} and was also inhibited by Cu^{2+}.

t. at page 127, paragraph 1

As evident from the results in Table 23, it was revealed that, after the action of the enzyme of the present invention, glucose and α-isomaltosylglucose alias 6^2-O-α-glucosylmaltose or panose were mainly formed from maltose as a substrate; and maltose and α-isomaltosylglucose alias 6^3-O-α-glucosylmaltotriose were mainly formed along with small amounts of glucose, maltotetraose, α-isomaltosylglucose alias 6^2-O-α-glucosylmaltose or panose, and the product X. Also, it was revealed that maltotriose and the product X were mainly formed from maltotetraose as a substrate along with small amounts of maltose, maltopentaose, α-isomaltosylglucose alias 6^3-O-α-glucosylmaltotriose; and the product Y; and that maltotetraose and the product Y were mainly formed from maltopentaose as a substrate along with small amounts of maltotriose, maltohexaose, and the products X and Z.

u. at page 128, paragraph 3-4

Based on these results, the product X formed from maltotetraose via the action of the α-isomaltosylglucosaccharide-forming enzyme of the present invention was revealed as a pentasaccharide, in which a glucose residue ~~bounds~~ binds via the α-linkage to OH-6 of glucose at the non-reducing end of maltotetraose, i.e., α-isomaltosylmaltotriose alias 6^4-O-α-glucosylmaltotetraose, represented by Formula 1.

Formula 1:

α-D-Glcp-(1 6)-α-D-Glcp-(1 4)-α-D-Glcp-(1 4)-α-D-Glcp-(1 4)-D-Glcp

The product Y formed from maltopentaose was revealed as a hexasaccharide, in which a glucosyl residue ~~bounded~~ binds via the α-linkage to OH-6 of glucose at the non-reducing end of maltopentaose, i.e., α-isomaltosylmaltotetraose alias 6^5-O-α-glucosylmaltopentaose, represented by Formula 2.

v. at page 132, paragraph 1

The following test was carried out to study whether the α-isomaltosylglucosaccharide-formation enzyme of the present invention had the ~~ability of forming~~ reducing power. To a 1% (w/v) aqueous solution of maltotetraose as a substrate was added 0.25 unit/g substrate, d.s.b., of either of purified specimens of α-isomaltosylglucosaccharide-forming enzyme from *Bacillus globisporus* C9 obtained by the method in Experiment 4-2, *Bacillus globisporus* C11 obtained by the method in Experiment 7-2, *Bacillus globisporus* N75 obtained by the method in Experiment 11-2, or *Arthrobacter globiformis* A19 obtained by the method in Experiment 15-2, and incubated at 35°C and pH 6.0, except that pH 8.4 was used for the enzyme from *Arthrobacter globiformis* A19. During enzymatic reaction, a portion of each reaction solution was sampled at prescribed time intervals and measured for reducing ~~powder~~ power after keeping the sampled solutions at 100°C for 10 min to suspend the enzymatic reaction. Before and after the enzymatic reaction, the reducing saccharide content and the total sugar content were respectively quantified by the Somogyi-Nelson's method and the anthrone-sulfuric acid reaction method.

w. at page 133, paragraph 2

To study whether the α-isomaltosylglucosaccharide-formation enzyme of the present invention has the ability ~~of forming~~to form dextran, it was tested in accordance with the method in *Bioscience Biotechnology and Biochemistry*, Vol. 56, pp. 169-173...

x. at page 134

To study whether the α-isomaltosylglucosaccharide-formation enzyme of the present invention has the ability of forming dextran, it was tested in accordance with the method in *Bioscience Biotechnology and Biochemistry*, Vol. 56, pp. 169-173 (1992). To a 1% (w/v) aqueous solution of maltotetraose as a substrate was added 0.25 unit/g substrate, d.s.b., of either of purified specimens of α-isomaltosylglucosaccharide-forming enzyme from *Bacillus globisporus* C9 obtained by the method in Experiment 4-2, *Bacillus globisporus* C11 obtained by the method in Experiment 7-2, *Bacillus globisporus* N75 obtained by the method in Experiment 11-2, or *Arthrobacter globiformis* A19 obtained by the method in Experiment 15-2, and incubated at 35°C and pH 6.0, except that pH 8.4 was used for the enzyme from *Arthrobacter globiformis* A19, for four or eight hours. After completion of the enzymatic reaction, the reaction was suspended by heating at 100°C for 15 min. Fifty microliters of each of the reaction mixtures were placed in a centrifugation tube and then admixed and sufficiently stirred with 3-fold volumes of ethanol, followed by standing at 4°C for 30 min. Thereafter, each mixture solution was centrifuged at 15,000 rpm for five minutes and, after removing supernatant, the resulting sediment was admixed with one milliliter of 75% (w/w) ethanol solution and stirred for washing. ~~The~~ Each resulting ~~each~~ solution was

centrifuged to remove supernatant, dried *in vacuo*, and then admixed and sufficiently stirred with one milliliter of deionized water. The total sugar content, in terms of glucose, of each resulting solution was quantified by the phenol-sulfuric acid method. As a control, the total sugar content was determined similarly as in the above except for using either of purified specimens of α-isomaltosylglucosaccharide-forming enzyme from *Bacillus globisporus* C9, *Bacillus globisporus* C11, *Bacillus globisporus* N75, and *Arthrobacter globiformis* A19, which had been inactivated at 100°C for 10 min...

y. at page 135, paragraph 1

As evident from the results in Table 27, it was revealed that the α-isomaltosylglucosaccharide-forming enzyme of the present invention did not substantially have the action of forming dextran or had only an undetectable level of such activity because it did not form dextran when it acted on maltotetraose.

z. at page 141, paragraph 2

The test on the formation of cyclotetrasaccharide by the α-isomaltosylglucosaccharide-forming enzyme and α-isomaltosyl-transferring enzyme was conducted using saccharides. For the test, ~~it was~~ prepared a solution of maltose, maltotriose, maltotetraose, maltopentaose, amylose, soluble starch, "PINE-DEX #100" (a partial starch hydrolyzate commercialized by Matsutani Chemical Ind., Tokyo, Japan), or glycogen from oyster commercialized by Wako Pure Chemical Industries Ltd., Tokyo, Japan was prepared.

aa. at page 144, paragraph 2

(1) The α-isomaltosylglucosaccharide-forming enzyme of the present invention acts on a glucose residue at the non-reducing end of an α-1,4 glucan chain of glycogen and partial starch hydrolyzates, etc., and intermolecularly ~~transfer~~ transfers the glucose residue to OH-6 of a glucose residue at the non-reducing end of ~~other~~ another α-1,4 glucan chain of glycogen to form an α-1,4 glucan chain having an α-isomaltosyl residue at the non-reducing end;

ab. at page 146, paragraph 1

A 15% corn starch suspension was prepared, admixed with 0.1% calcium carbonate, adjusted to pH 6.0, and then mixed with 0.2-2.0% per gram starch of "TERMAMYL 60L", an α-amylase specimen commercialized by Novo Indutri A/S, Copenhagen, Denmark, followed by the enzymatic reaction at 95°C for 10 min. Thereafter, the reaction mixture was autoclaved at 120°C for 20 min, promptly cooled to about 35°C to obtain a liquefied starch with a DE (dextrose equivalent) of 3.2-20.5. To the liquefied starch were added two units/g solid of a purified specimen of α-isomaltosylglucosaccharide-forming enzyme from Strain C11 obtained by the method in Experiment 7-2, and 20 units/g solid of a purified specimen of α-isomaltosyl-transferring enzyme from Strain C11 obtained by the method in Experiment 7-3, followed by the incubation at 35°C for 24 hours. After completion of the

reaction, the reaction mixture was heated at 100°C for 15 min to inactivate the remaining enzymes. Then, the reaction mixture thus obtained was treated with α-glucosidase and glucoamylase similarly as in Experiment 1 to hydrolyze the remaining reducing oligosaccharides, followed by quantifying the formed cyclotetrasaccharide on HPLC. The results are in ~~Tale~~ Table 31.

ac. at page 148, paragraph 2

As is evident from ~~he~~ the results in Table 32, the formation yield of cyclotetrasaccharide was about 64% at a low concentration of 0.5%, while it was about 40% at a high concentration of 40%. The fact indicated that the formation yield of cyclotetrasaccharide increased depending on the concentration of partial starch hydrolyzate as a substrate. The result revealed that the formation yield of cyclotetrasaccharide increased as the decrease of partial starch hydrolyzate.

ad. at page 151, paragraph 1

The saccharide solution was fed to a column packed with about 225 L of "AMBERLITE CR-1310 (Na-form)", an ion-exchange resin commercialized by Japan Organo Co., Ltd., Tokyo, Japan, and chromatographed at a column temperature of 60_C and a flow rate of about 45 L/h. While the saccharide composition of eluate from the column was ~~monitoring~~ monitored by HPLC as described in Experiment 1, fractions of cyclotetrasaccharide with a purity of at least 98% were collected, and in a usual manner desalted, decolored, filtered, and concentrated to obtain about 7.5 kg of a saccharide solution with a solid content of about 2,500 g solids. HPLC measurement for saccharide composition of the

saccharide solution revealed that it contained cyclotetrasaccharide with a purity of about 99.5%.

ae. page 152, paragraph 2

The thermogravimetric analysis of the cyclotetrasaccharide in a crystalline form gave a thermogravimetric curve in FIG. 47. Based on the relationship between the weight change and the temperature, it was successively found that the weight reduction corresponding to four or five moles of water was observed up to a temperature of 150°C, the weight reduction corresponding to one mole of water at around 250°C, and the weight reduction corresponding to the decomposition of cyclotetrasaccharide at a temperature of about 280°C or higher. These results confirmed that the cyclotetrasaccharide crystal, penta- or hexa-hydrate, of the present invention releases four or five moles of water to changes into a monohydrate crystal when heated up to 150°C at normal pressure, and further releases one mole of water to change into an anhydrous crystal ~~until~~ after being heated up to 250°C.

af. at page 154

Cyclotetrasaccharide, penta- or hexa-hydrate, in a crystalline powder form, obtained by the method in Experiment 30, was dried *in vacuo* at 40°C or 120°C for 16 hours. The Karl Fischer method of the resulting crystalline powders revealed that the one dried at 40°C had a moisture content of about 4.2%, while the other dried at 120°C had a moisture content of about 0.2%, meaning that it was substantially anhydrous. Unlike quite different from the results from powder x-ray diffraction analyses of the cyclotetrasaccharide, penta- or hexa-hydrate, and the

cyclotetrasaccharide, monohydrate, before drying *in vacuo*, the powder x-ray analysis of the above cyclotetrasaccharide dried *in vacuo* at 40° and 120°C gave characteristic diffraction spectra having main diffraction angles (2θ) of 10.8°, 14.7°, 15.0°, 15.7°, and 21.5° in FIG. 50 for 40_C and FIG. 51 for 120°C. Although there found difference in peak levels between the two diffraction spectra, they had substantially the same peak diffraction angles and they were crystallographically judged to be substantially the same crystalline monohydrate. The fact that the base lines of the diffraction spectra exhibited a mountain-like pattern and the crystallinity of the crystalline monohydrate was lower than those of cyclotetrasaccharide, penta- or hexa-hydrate, and cyclotetrasaccharide, monohydrate, before drying *in vacuo* revealed that there existed an amorphous cyclotetrasaccharide. Based on this, the cyclotetrasaccharide powder with a moisture content of about 4.2%, obtained by drying *in vacuo* at 40°C, was estimated to be a mixture powder of an amorphous cyclotetrasaccharide with such a moisture content and anhydrous crystalline cyclotetrasaccharide. These data revealed that cyclotetrasaccharide, penta- or hexa-hydrate, was converted into ~~those in an~~ amorphous and anhydrous forms when dried *in vacuo*. The thermogravimetric analysis of anhydrous cyclotetrasaccharide with a moisture content of 0.2%, which was conducted similarly as in Experiment 31, observed only a weight reduction as shown in FIG. 52, deemed to be induced by the heat decomposition at a temperature of about 270°C or higher as shown in FIG. 52.

ae. <u>at page 155, paragraph 1</u>

To study the saturation concentration of cyclotetrasaccharide in water at 10-90°C, 10 ml of water was placed in a glass vessel with a seal cap, and then mixed with

cyclotetrasaccharide, penta- or hexa-hydrate, obtained by the method in Experiment 30, in an excessive amount over a level dissolving completely at respective temperatures, cap-sealed, and stirred for two days while keeping at respective temperatures of 10-90°C until being saturated. ~~The~~ Each resulting ~~each~~ saturated solution of cyclotetrasaccharide was membrane filtered to remove undissolved cyclotetrasaccharide, and each filtrate was then examined for moisture content by the drying loss method to determine a saturation concentration of cyclotetrasaccharide at respective temperatures. The results are in Table 34.

ah. at page 159, paragraph 1

A crystalline cyclotetrasaccharide, penta- or hexa-hydrate, obtained by the method in Experiment 30, and a commercialized polypeptone, Nihonseiyaku K.K., Tokyo, Japan, were dissolved in deionized water to obtain a 10% (w/v) cyclotetrasaccharide solution containing 5% (w/v) polypeptone. Four milliliters of the resulting solution were placed in a glass test tube, sealed, and heated at 100°C for 30 to 90 min. After allowing to stand for cooling at ambient temperature, each of the resulting ~~solution~~ solutions was measured for coloration degree to examine on their amino carbonyl reactivity. In parallel, as a control, a solution with only polypeptone was provided and similarly treated as above. The coloration degree was evaluated based on the level of the absorbance, measured in a cell with 1-cm light pass at a wavelength of 480 nm, minus the ~~one of the~~ control. The results are in Table 38.

ai. at page 165, paragraph 2

The acute toxicity of a crystalline cyclotetrasaccharide, penta- or hexa-hydrate, obtained by the method in Experiment 30, was tested by orally administering it to mice. As a result, it was revealed that cyclotetrasaccharide had relatively low toxicity and did not induce death of mice even when administered at a highest possible dose. Based on this, the LD_{50} of cyclotetrasaccharide was at least 50 g/kg mouse body weight, though the data ~~was~~ were not so accurate.

aj. at page 179, paragraph 2

To 0.8 part by weight of a crystalline tetrasaccharide, penta- or hexa-hydrate, obtained by the method in Example A-7, were homogeneously added 0.2 part by weight of "TREHA ", a crystalline trehalose hydrate commercialized by Hayashibara Shoji Inc., Okayama, Japan, 0.01 part by weight of "αG SWEET™" (α-glycosylstevioside commercialized by Toyo Sugar Refining Co., Tokyo, Japan), and 0.01 part by weight of "~~ASPALTAME~~ASPARTAME" (L-aspartyl-L-phenylalanine methyl ester), and the resulting mixture was fed to a granulator to obtain a sweetener in a granule form. The product has a satisfactory sweetness and a 2-fold higher sweetening power of sucrose. Since crystalline cyclotetrasaccharide, penta- or hexa-hydrate, is scarcely digested and fermented and is substantially free of calorie, the calorie of the product is about 1/10 of that of sucrose with respect to sweetening power. In addition, the product is substantially free from deterioration and stable when stored at ambient temperature. Thus, the product can be suitably used as a high quality low-caloric and less cariogenic sweetener.

ak. at page 184, paragraph 2

Ten parts by weight of beans as a material in a usual manner were boiled in a usual manner after the addition of water, removed the astringency, lye, and water-soluble impurities to obtain about 21 parts by weight of raw bean jam in the form of a granule. To the raw bean jam ~~of~~ were added 14 parts by weight of sucrose, five parts by weight of a syrup containing cyclotetrasaccharide, obtained by the method in Example A-3, and four parts by weight of water, and the resulting mixture was boiled, admixed with a small amount of salad oil, and then kneaded up without pasting the beans to obtain about 35 parts by weight of the desired product, *an*. Since the product has a satisfactory stability, mouth feel, taste, and flavor, and does not substantially exhibit~~has~~ syneresis and excessive color ~~of~~ upon baking, it can be arbitrarily used as a material for confectioneries such as a bean jam bun, "*manju*" (a kind of Japanese confectionery with bean jam), bean-jam-filled wafer, and ice cream/candy.

al. at page 187, paragraph 4

The product is a high quality bath salt enriched with *yuzu* flavor and used by diluting in hot water by 100-10,000 folds, and it moisturizes and smooths the skin and does not make ~~you~~ one feel cold after taking a bath therewith.

am. at page 191, paragraph 3 and at page 192, paragraph 1

As described above, the present invention relates to a novel α-isomaltosylglucosaccharide-forming enzyme, and their process and uses, more particularly, to a novel α-

isomaltosylglucosaccharide-forming enzyme, process thereof, microorganisms producing the enzyme, α-glucosyl-transferring method using the enzyme, a method for forming α-isomaltosylglucosaccharide, a process for producing cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→}, and a composition comprising the saccharide obtainable therewith. According to the present invention, an industrially useful cyclotetrasaccharide having the structure of cyclo{66)-α-D-glucopyranosyl-(163)-α-D-glucopyranosyl-(166)-α-D-glucopyranosyl-(163)-α-D-glucopyranosyl-(16} or a composition comprising the same can be produced on an industrial scale and at a relatively low cost. Since these cyclotetrasaccharides~~cyclotetrasaccharide~~ and the saccharide comprising the same have substantially no or low reducibility, substantially do not cause the amino carbonyl reaction, substantially do not exhibit hygroscopicity, have easily handleability, have mild sweetness, adequate viscosity, moisture-retaining ability, inclusion ability, and substantially no digestibility, they~~it~~ can be advantageously used in a variety of compositions such as food products, cosmetics, pharmaceuticals as a sweetener, material for low caloric foods, taste-improving agent, flavor-improving ability, quality-improving agent, syneresis-preventing agent, stabilizer, filler, inclusion agent, and base for pulverization. The present invention, having these outstanding functions and effects, is a significantly important invention that greatly contributes to this art.